AFM imaging reveals the assembly of a P2X receptor complex containing P2X2, P2X4 and P2X6 subunits

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protein yielded similar results. However, sedimentation equilibrium analysis yielded dissociation constants of 160 to 280 nM. Temperature and pressure variations did not explain this difference. Small amounts of degradation over the several day sedimentation equilibrium protocol was revealed by silver-stained SDS-PAGE and likely underlies the different Kds for sedimentation velocity and sedimentation equilibrium experiments. Our results confirm that the GluA2 ATD forms nM affinity dimers3. The spread of values measured by SV highlights the difficulty in making accurate measurements at nanomolar protein concentrations, due to dimer-deficient monomers produced by proteolysis or extremely small signal to noise ratios. The similar variance observed in prior work with fluorescence detection AUC suggests that S/N limitations are not the major cause of Kd variations.

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A Novel Toxin that Targets Acid-Sensing Ion Channels to Produce Pain

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Natural products have served as invaluable keys to unlocking molecular underpinnings of pain sensation. In particular, plant-derived irritants, such as capsaicin, menthol, mustard oil, have proven essential for identifying and/or characterizing excitatory TRP ion channels that play major roles in pain sensation. Animals also possess chemical defense mechanisms to inflict pain in predators. To more fully exploit the rich pharmacopeia of animal toxins, we established an unbiased screen to discover venoms capable of activating specific subpopulations of somatosensory neurons, with the goal of developing novel probes and identifying important physiological targets for pain research and therapeutics. We show that venom from the Texas coral snake - whose bite produces excruciating pain - contains a novel toxin that potently and persistently activates a subset of somatosensory neurons. First, we use biochemical and molecular methods to identify a unique two-component toxin that form a high affinity heteromeric complex. Second, we use electrophysiological and genetic approaches to identify acid-sensing ion channels (ASICs) as the molecular target. Furthermore, behavioral experiments show that toxin-evoked activation of ASIC channels elicits pain by recruiting a canonical population of primary afferent nociceptors that detect thermal and inflammatory pain.

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Conformational Change Involved in Gating of Acid Sensing Ion Channel (ASIC1a)

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Acid sensing ion channels are proton activated, sodium selective channels and are trimeric in nature. They belong to the epithelial sodium channel family. They are widely expressed in peripheral sensory neurons and neurons of central nervous system. These channels are involved in various physiological functions like sodium homeostasis, pain, mechano sensation, acidosis induced neuronal injury, etc. Here we expressed a modified chicken acid sensing ion channel (ASIC1a) in insect cells containing engineered cysteine residues at positions 129 and 338, at the finger and thumb domain respectively. The membrane preparations of the protein expressed in insect cells was shown to be functional using bilayer measurements. Using this functional construct we performed Fluorescence Resonance Energy Transfer measurements by tagging the insect cells with terbium chelate as donor and ATTO 465 as the acceptor. The distance between the donor and acceptor was similar to that observed in crystal structure thus indicating that the crystal structure is a good representation of the functional receptor in the membranes. Additionally, decreasing the pH from 7.4 to 6 resulted in a decrease in distance between the two residues, consistent with the movement of the thumb domain closer to the finger domain as previously hypothesized based on the crystal structure.

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Modulation of Human Acid-Sensing Ion Channel 1A Open Channel Inactivation by FRRFamide

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The ASICs (Acid-Sensing Ion Channels) are involved in neuronal signaling in the central and peripheral nervous system. These non-voltage-gated channels are involved in learning, the expression of fear, neurodegeneration after ischemia, and pain sensation. The molecular bases underlying their activity are not yet fully understood. During an extracellular acidification, ASICs open transiently before inactivating in the continued presence of the low extracellular pH. Modulators of ASIC inactivation may contribute to the physiological and pathological functions of ASICs. FRRFamide (FRRFa) and related peptides have been shown to slow the ASIC inactivation time course and to induce a small sustained current. In the present study we have carried out in silico docking of FRRFa to human ASIC1a, which predicted two cavities as FRRFa binding site. It has previously been shown that a part of the thumb region differs between ASIC1 orthologs, and that FRRFa induces greater sustained currents in human than in mouse ASIC1a, suggesting that this region may be involved in the effect of FRRFa.

The role of the residues predicted to be part of the FRRFa binding site (one of the top docking poses) has been tested by site-directed mutagenesis and functional studies. While mutation of only one residue of the predicted binding site decreased the effect of FRRFa, several point mutations in the β9-α4 region increased the FRRFa-induced sustained current. Our results indicate that the β9-α4 region is likely not the FRRFa binding site, however that it is involved in the effect of FRRFa on ASIC inactivation.

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AFM Imaging Reveals the Assembly of a P2X Receptor Complex Containing P2X2, P2X4 and P2X6 Subunits

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Seven P2X purinergic receptor subunits have been identified: P2X1-P2X7. All except P2X6 assemble as homotrimers, and six heteromeric receptors (P2X1/2, P2X1/4, P2X1/5, P2X2/3, P2X2/6 and P2X4/6) have been described. In addition, P2X4 homomers associate with P2X2 or P2X7 homomers as dimers of trimers. The various P2X receptors show individual functional properties, suggesting distinct physiological roles. The overlapping expression of P2X2, P2X4 and P2X6 subunits has been shown in different cell types, and functional analysis of P2X receptors in Leydig cells suggests that the three subunits interact. In the present study we investigated the potential assembly of P2X2, P2X4 and P2X6 subunits into heteromeric receptors. tsA 201 cells were co-transfected with His6-tagged P2X2, HA-tagged P2X4 and FLAG-tagged P2X6 subunits. After sequential co-immunoprecipitation using anti-HA and anti-FLAG resins, all three subunits were present, demonstrating their interaction. Proteins eluted from the resins were incubated with anti-His6 antibodies and anti-HA Fab fragments, and analyzed by atomic force microscopy (AFM). In 292 AFM images, 23 central particles with volumes expected for P2X trimers were found to be doubly decorated by one antibody and one Fab fragment. In contrast, only two such complexes were seen when the antibody/Fab incubation was omitted (223 images). Two complexes were also seen after incubation with anti-Myc antibodies plus anti-V5 Fab fragments (control; 263 images). This result is consistent with the presence of a P2X2/4/6 heterotrimer.

We conclude that P2X2, P2X4 and P2X6 subunits interact, potentially forming a heterotrimeric receptor containing three different subunits.

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Subtype Specific Activation of P2X Receptors by Free and Magnesium-Bound ATP

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P2X receptors are cation-selective channels that were suggested to play roles in many important physiological processes, including muscle contraction, pain sensation, and inflammation. Extracellular ATP released from various sources, such as synaptic vesicles and damaged cells, is the ligand for activating P2X receptors. In neutral solution, ATP is ionized and exists mostly as free ATP (ATP^{4-}), a high affinity chelator for divalent and trivalent cations. As there